

amendments and sequence listing provided herein and in supporting documents, including the CRF.

Applicants believe that the present response is a bona fide attempt to advance the application, and is substantially a complete reply to the Notice to Comply with Requirements For Patent Applications Containing Nucleotide sequence and/or Amino Acid Sequence Disclosures mailed August 05, 2002. Therefore, if consideration of some matter or compliance with some requirement has been inadvertently omitted, Applicants respectfully request that the Examiner provide a new time period for reply under 37 C.F.R. § 1.134 to supply the omission.

The Examiner is requested to contact the representative for the Applicants, to discuss any questions or for clarification.

No further fee is believed necessary. If any further fee associated with this Response is required, the Director is authorized to charge our Deposit Account No. 19-0962.

Respectfully submitted,

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Date

  
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## APPENDIX A

### VERSION WITH MARKINGS TO SHOW CHANGES MADE

The paragraph starting at page 10, line 3 is amended as follows:

Figure 5 illustrates the dicistronic expression vector, pComb, in the form of a phagemid expression vector. The amino acid residue sequence of the decapeptide tag and the Pel B leader sequence/spacer are listed in SEQ ID NOS: 5 and 6, respectively. The amino acid residue sequence of the leader sequence in the lower panel is listed in SEQ ID NO:7.

The paragraph starting at page 16, line 16, is amended as follows:

Figures 25A and 25B illustrate the nucleotide and amino acid residue sequences of the b12 light chain gene in the pSG-5 mammalian expression vector described in Example 4b. The b12 light chain has been modified for expression in mammalian cells as described in Example 4b. The upper and lower strands of the nucleotide sequences correspond to SEQ ID NO:152 and SEQ ID NO:168 in the sequence listing, respectively. The amino acid residue sequence corresponds to SEQ ID NO:153 in the sequence listing.

The paragraph starting at page 16, line 32, is amended as follows:

Figures 27A through 27E illustrate the nucleotide and amino acid residue sequences of the b12 heavy chain VH and constant regions in the pEe6HC BM12 mammalian expression vector as

described Example 4d. The b12 VH has been modified for expression in mammalian cells as described in Example 4d. The upper and lower strands of the nucleotide sequences correspond to SEQ ID NO:154 and SEQ ID NO:169 in the sequence listing, respectively. The amino acid residue sequence corresponds to SEQ ID NO:155 in the sequence listing.

The paragraph starting at page 17, line 23, is amended as follows:

Figure 29A through 29[S]R illustrates the nucleotide sequence of the pEE12 mammalian expression vector and the b12 IgG1 heavy and light chain genes, pEe12 Combo BM 12, as described in Example 4f. The VH and light chain genes have been modified for expression in mammalian cells as described in Example 4. The upper and lower strands of the nucleotide sequences correspond to SEQ ID NO:156 and SEQ ID NO:170 in the sequence listing, respectively.

The paragraph starting at page 34, line 35, is amended as follows:

Particularly preferred is the immunoglobulin IgG1 human antibody described herein that is comprised of the b12 antibody Fab fragment and human Fc domain derived from an IgG1 subtype, designated b12 IgG1. The structure and preparation of this preferred human monoclonal antibody is described herein, and is prepared using the recombinant DNA expression vector pEE12. The complete nucleotide sequence of the vector for expression the complete heavy and light chains in the form of b12 IgG1 is shown

in Figure 27 and also in SEQ ID NOs [156]154 and [170]169. Accordingly, the amino acid residue and nucleotide sequences, respectively, for a preferred complete heavy chain are shown in SEQ ID NOs 155 and 154, respectively, and for a preferred light chain are shown in SEQ ID NOs 153, and 152, respectively. The nucleotide sequences for preferred heavy and light chains are also shown in SEQ ID NOs 169 and 168, respectively.

The paragraph starting at page 121, line 8, is amended as follows:

First, the b12 VH region was cloned into a pSG-5 expression vector (Green et al., Nucl. Acids Res., 16:369 (1988)) to fuse the b12 VH to the heavy chain constant domains (CH1, CH2, and CH3) of an IgG1 antibody molecule. The double-stranded Fab b12 DNA was used as a template for isolating the gene encoding the VH region of the Fab b12, the amino acid residue sequence of which is listed in SEQ ID NO 66. Fab b12 DNA and mouse B73.2 IgG1 DNA (Whittle, et al., Protein Eng., 1:499 (1987) and Bruggeman, et al., J. Exp. Med., 166:1351 (1987)) were used as templates for a PCR amplification for the construction of a DNA fragment consisting of the unique Kozak sequence for the control of heavy chain expression, the mouse B72.3 heavy chain leader sequence (MEWSWVFLFFLSVTTGVHS [(SEQ ID NO 155 from amino acid residue sequence 1 to 20)](SEQ ID NO 171)), the human VH consensus sequence (QVQLVQ [(SEQ ID NO 155 from amino acid residue sequence 21 to 26)](SEQ ID NO 172)), and the VH region of the Fab b12. Altering the beginning of the VH from the mouse consensus sequence to the human consensus sequence also destroyed the original Xho I cloning site. The restriction sites EcoR I

and Sst I were introduced in the amplification reaction and were located at the 5' and 3' ends of the fragment, respectively. The procedure for creating the modified VH fragment by combining the products of the two separate PCR amplifications is described below.

The paragraph starting at page 129, line 7, is amended as follows:

The b12 light chain was cloned into a separate pSG-5 expression vector (Green et al., supra). The double-stranded Fab b12 DNA was used as a template for isolating the gene encoding the light chain of the Fab b12, the amino acid residue sequence of the light chain of Fab b12 is listed in SEQ ID NO 97. Mouse B73.2 IgG1 DNA (Whittle, et al., Protein Eng., 1:499 (1987) and Bruggeman, et al., J. Exp. Med., 166:1351 (1987)) was used as a template for isolating the mouse B73.2 leader sequence. Fab b12 and mouse B73.2 IgG1 DNA were thus used as templates for a PCR amplification for the construction of a DNA fragment consisting of the unique Kozak sequence for control of light chain expression, the mouse B72.3 light chain leader sequence (MGVPTQLGLLLWLTDARC [(SEQ ID NO 153 from amino acid residue sequence 1 to 20)] (SEQ ID NO 173)), and the b12 light chain beginning with a human light chain amino acid consensus sequence (EIVLTQSP [(SEQ ID NO 153 from amino acid residue sequence 21 to 28)] (SEQ ID NO 174)). Altering the beginning of the light chain from the mouse amino acid consensus sequence to the human amino acid consensus sequence also destroys the original Sac I cloning site. The restriction site, EcoR I, was introduced in the amplification reactions and was located at both the 5' and 3'

ends of the fragment. The procedure for creating this fragment by combining the products of two separate PCR amplifications is described below.

The paragraph starting at page 137, line 19, is amended as follows:

The heavy chain cassette was removed from the pEE6 vector by digestion with BglII and Sal I. The pEE12 vector containing the light chain gene, prepared in Example 4e, was also digested with BglII and Sal I. The heavy chain cassette and the pEE12 vector containing the light chain gene from Example 4e were ligated together at the BglII and Sal I sites as described in Example 4d. The combinatorial construct was transformed into DH5 $\alpha$  competent cells and miniprep DNA was analyzed for the presence of the heavy and light chains as in Example 4d. The nucleotide sequence of the heavy and light chain genes was determined. The nucleotide sequence of pEe12 Combo BM 12, the pEE12 vector containing the b12 heavy and light chain genes is given in the sequence listing as SEQ ID NO 156 and is illustrated in Figures 29A through 29[S]R.

The paragraph starting at page 165, line 30, is amended as follows:

Infectious culture supernatants containing virus and free gp120 were treated with 1% (v/v) Nonidet-P40 (NP40) non-ionic detergent to provide a source of gp120 (Moore et al., AIDS, 3:155-160 (1989)). Microplate wells (Immulon II, Dynatech, Ltd.) were first coated with sheep polyclonal antibody D7324. This

antibody was raised to the peptide APTKAKRRVVQREKR (SEQ ID NO:175), derived from the C-terminal 15 amino acids of the clade B IIIB HIV-1 viral isolate. Next, an appropriate volume of inactivated supernatant containing gp120 was diluted with a buffer comprising tris-buffered saline (TBS)/1% (v/v) NP40/10% fetal calf serum (FCS) and a 100  $\mu$ l aliquot added to the microplate wells for 2 hours at room temperature. Unbound gp120 was removed by washing with TBS, and bound gp120 was detected with CD4-IgG (1  $\mu$ g/ml) or with b12 IgG1 diluted in a buffer comprising TBS/2% (w/v) nonfat dry milk powder/20% (v/v) sheep serum (TMTSS) essentially as previously described (Moore et al., AIDS, 4:307-310 (1990)) and Moore et al., J. Virol., 68:469-473 (1994)). CD4-IgG is a fusion molecule which consists of CD4 and IgG. The CD4 portion binds to gp120 and the IgG portion provides the means for detection of the CD4-IgG fusion molecule with labeled anti-IgG reagents. Bound antibody was then detected with an appropriate alkaline-phosphatase conjugated anti-IgG, followed by AMPAK (Dako Diagnostics). Absorbance was determined at 492 nm (OD<sub>492</sub>). Each virus was tested against CD4-IgG in triplicate and against b12 IgG1 in duplicate. All OD<sub>492</sub> values were corrected for non-specific antibody binding in the absence of added gp120 (buffer blank). The mean, blank-corrected OD<sub>492</sub> values for CD4-IgG and b12 IgG1 were then calculated, and the OD<sub>492</sub> ratios of b12 IgG1:CD4-IgG were determined. This normalization procedure enables allowance to be made for the different amounts of gp120 captured onto the solid phase via antibody D7324 when comparing antibody reactivity with a panel of viruses. Binding ratios of 0.50 or greater were deemed to represent strong antibody reactivity; ratios from 0.25-0.50 were considered indicative of moderate reactivity; values of <0.25 were designated as

representative of essentially negative monoclonal antibody reactivity.

The paragraph starting at page 184, line 26, is amended as follows:

A binary plasmid system consisting of two replicon-compatible plasmids was constructed as shown in 14. the pTAC01H heavy chain vector schematic is shown in figure 14A and the pTC01 light chain vector schematic is shown in Figure 14B. Both expression vectors feature similar cloning sites including pel B leader sequences fused to the ribosome binding sites and the tac promoters via BamH I sites as shown in Figure 15. The nucleotide sequences of the multiple cloning sites along with the tac promoter, ribosome binding sites (rbs) and the underlined relevant restriction sites for the light chain vector, pTC01, and heavy chain vector, pTAC01H, are respectively shown in Figure 15A and Figure 15B. The sequences are also listed in the Sequence Listing aas described in the Brief Description of the Drawings. The heavy chain vector pTAC01H also contains a (His)<sub>5</sub>[-](SEQ ID NO:176) tail to allow purification of the recombinant Fab fragments by immobilized metal affinity chromatography. The presence of both plasmids in the same bacterial cell is selected for by the presence of both antibiotics in the media. Expression is partially suppressed during growth by addition of glucose and induced by the addition of IPTG at room temperature. Under these conditions, both plasmids are stable within the cell and support expression of the Fab fragment as assayed by ELISA using goat anti-human kappa and goat anti-human IgG1 antibodies.